Multiple Regulatory Elements for the *glpA* Operon Encoding Anaerobic Glycerol-3-Phosphate Dehydrogenase and the *glpD* Operon Encoding Aerobic Glycerol-3-Phosphate Dehydrogenase in *Escherichia coli*: Further Characterization of Respiratory Control

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In *Escherichia coli*, *sn*-glycerol-3-phosphate can be oxidized by two different flavo-dehydrogenases, an anaerobic enzyme encoded by the *glpACB* operon and an aerobic enzyme encoded by the *glpD* operon. These two operons belong to the *glp* regulon specifying the utilization of glycerol, *sn*-glycerol-3-phosphate, and glycerophosphodiester. In *glpR* mutant cells grown under conditions of low catabolite repression, the *glpA* operon is best expressed anaerobically with fumarate as the exogenous electron acceptor, whereas the *glpD* operon is best expressed aerobically. Increased anaerobic expression of *glpA* is dependent on the *fnr* product, a pleiotropic activator of genes involved in anaerobic respiration. In this study we found that the expression of a *glpA1*(Oxr) (oxygen-resistant) mutant operon, selected for increased aerobic expression, became less dependent on the FNR protein but more dependent on the cyclic AMP-catabolite gene activator protein complex mediating catabolite repression. Despite the increased aerobic expression of *glpA1*(Oxr), a twofold aerobic repressibility persisted. Moreover, anaerobic repression by nitrate respiration remained normal. Thus, there seems to exist a redox control apart from the FNR-mediated one. We also showed that the anaerobic repression of the *glpD* operon was fully relieved by mutations in either *arcA* (encoding a presumptive DNA recognition protein) or *arcB* (encoding a presumptive redox sensor protein). The *arc* system is known to mediate pleiotropic control of genes of aerobic function.

Glycerol is utilized by *Escherichia coli* obligatorily through phosphorylation to glycerol-3-phosphate (G3P), which can also be captured directly from the environment or after periplasmic hydrolysis of glycerophosphodiester (18, 19). G3P is oxidized by either the anaerobic dehydrogenase encoded by the *glpACB* operon (min 49) or the aerobic dehydrogenase encoded by the *glpD* operon (min 75) (Fig. 1). These two operons, together with the *glpFK* encoding glycerol kinase and glycerol facilitator (min 88) and the *glpTQ* operons (min 49) encoding G3P permease and glycerophosphodiesterase, are members of the *glp* regulon specifically controlled by the *glpR*-encoded repressor. The *glpACB* and the *glpTQ* operons are adjacent to each other but are divergently transcribed (3, 4, 14, 18–20; G. Sweet, personal communication). Two structural genes of the same regulon but of unknown function, *glpE* and *glpG*, were found between *glpD* and *glpR* (22).

With a *glpR* deletion mutant, Freedberg and Lin (5) observed the highest activity level of the *glpD* enzyme after aerobic growth on casein acid hydrolysate and the highest activity level of the *glpA* enzyme after anaerobic growth on casein hydrolysate plus fumarate. Submaximal activity levels of both enzymes were observed when nitrate served as the exogenous electron acceptor. Since catabolite repression could not account for the pattern of these variations, a respiratory control mechanism was invoked (5). Kuritzkes et al. (16) subsequently discovered that the increased anaerobic

expression of the *glpA* operon was dependent on the *fnr* product, a pleiotropic activator of genes involved in anaerobic respiration (for a review, see reference 26). In this study we further explored the respiratory control of *glpA* and *glpD* expression.

MATERIALS AND METHODS

Materials. Phenazine methosulfate, 3-(4,5-dimethylthiazolyl-2-)-2,5-diphenyl tetrazolium bromide (MTT), benzyl viologen, *o*-nitrophenyl- β -galactopyranoside, and DL-dithiothreitol were obtained from Sigma Chemical Co., St. Louis, Mo.; 5-bromo-4-chloro-3-indolyl- β -galactopyranoside was obtained from Bachem, Inc., Torrance, Calif.; [U - ^{14}C]G3P, disodium salt, and Biofluor were obtained from Dupont, NEN Research Products, Boston, Mass.; MacConkey medium, tryptone, and yeast extract were obtained from Difco Laboratories, Detroit, Mich.; and vitamin-free casein acid hydrolysate was obtained from ICN Nutritional Biochemicals, Cleveland, Ohio. All other reagents used were commercial products of the highest grade available.

Bacterial and phage strains. All strains used were derivatives of *E. coli* K-12. Their origins and genotypes are given in Table 1. P1 vir phage were used for transductions. The Φ [*glpA1*(Oxr)-*lac*] locus (Oxr, oxygen resistant) was transduced from strain ECL503 to strain ECL514 by selection for aerobic growth on lactose mineral agar. The *glpR* mutation was transduced from strain ECL72 to strains ECL392 and ECL519 by selecting for the closely linked *glpD*⁺ marker which conferred aerobic growth ability on glycerol (20 mM)

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FIG. 1. The dissimilatory system for glycerol, G3P, and glycerophosphodiester in *E. coli* encoded by the *glp* regulon. The genetic symbols are in parentheses. Dashed arrows indicate feedback inhibitors of glycerol kinase activity: enzyme III^{Glc}, the protein III of the phosphoenolpyruvate phosphotransferase system for the vectorial phosphorylation of glucose, and fructose-1,6-P₂, fructose-1,6-bisphosphate. The dotted arrow indicates the pathway for G3P biosynthesis. Positions of the *glp* genes and operons (arrows over genetic symbols show the directions of transcriptions) are indicated on the circular figure (for a review, see reference 19).

mineral agar. The *fnr* mutation in strains ECL538 and ECL539, the *chlE* mutation in strain ECL566, the *arcA* mutation in strain ECL597, and the *arcB* mutation in strain ECL598 were acquired as nonselective markers together

with the linked Tn10 by selection on tetracycline-LB agar. The *narH200::Tn10* in strain ECL392 was selected for tetracycline resistance. All transductants were purified on agar with the same composition as that used for the selection, and

TABLE 1. *E. coli* strains used in this study

Strain	Genotype ^a	Derivation	Source or reference
RK5263	<i>narH200::Tn10</i>		28
ECL72	<i>glpR</i>		5
ECL323	<i>fnr-1 zci::Tn10</i>		16
ECL389	F ⁻ Φ (<i>glpA-lac</i>) Δ <i>glpD102</i> <i>recA</i> <i>srl::Tn10</i> <i>araD139</i> Δ (<i>argF-lac</i>) <i>U169 deoC1 gal flb-5301 ptsF relA1 rpsL150 sdh-9</i>		8
ECL392	F ⁻ Φ (<i>glpA-lac</i>) Δ <i>glpD102</i> <i>araD139</i> Δ (<i>argF-lac</i>) <i>U169 deoC1 gal flb-5301 ptsF relA1 rpsL150 sdh-9</i>		8
ECL503	Φ [<i>glpA1</i> (Oxr)- <i>lac</i>] <i>frd-1</i> (Con)		8
ECL514	F ⁻ Φ (<i>glpA-lacZ</i> ⁺ <i>Y</i>) Δ <i>glpD102</i> <i>araD139</i> Δ (<i>argF-lac</i>) <i>U169 deoC1 gal flb-5301 ptsF relA1 rpsL150 sdh-9</i>		9
ECL519	Φ [<i>glpA1</i> (Oxr)- <i>lac</i>] Δ <i>glpD102</i>	P1(ECL503) $\times$ ECL514	This study
ECL526	Φ (<i>glpA-lac</i>) <i>glpR</i>	P1(ECL72) $\times$ ECL392	This study
ECL527	Φ [<i>glpA1</i> (Oxr)- <i>lac</i>] <i>glpR</i>	P1(ECL72) $\times$ ECL519	This study
ECL538	Φ (<i>glpA-lac</i>) <i>glpR fnr-1 zci::Tn10</i>	P1(ECL323) $\times$ ECL526	This study
ECL539	Φ [<i>glpA1</i> (Oxr)- <i>lac</i>] <i>glpR fnr-1 zci::Tn10</i>	P1(ECL323) $\times$ ECL527	This study
ECL560	Φ (<i>glpA-lac</i>) <i>narH200::Tn10</i> Δ <i>glpD102</i>	P1(RK5253) $\times$ ECL392	This study
ECL562	<i>chlE103 zbi-624::Tn10</i> Δ <i>glpD102</i>		10
ECL565	Φ (<i>glpA-lac</i>) <i>narL215::Tn10</i> Δ <i>glpD102</i>		11
ECL566	Φ (<i>glpA-lac</i>) <i>chlE103 zbi-624::Tn10</i> Δ <i>glpD102</i>	P1(ECL562) $\times$ ECL392	This study
ECL585	<i>arcA1 zji::Tn10</i>		12
ECL594	<i>arcB1 zgi::Tn10</i>		7
ECL597	Φ (<i>glpA-lac</i>) <i>glpR arcA1 zji::Tn10</i>	P1(ECL585) $\times$ ECL526	This study
ECL598	Φ (<i>glpA-lac</i>) <i>glpR arcB1 zgi::Tn10</i>	P1(ECL594) $\times$ ECL526	This study

^a Φ (*glpA-lac*) refers to the fusion *glpA101:: λ p1*(209) (8); Φ (*glpA-lacZ*⁺*Y*) refers to the stabilized *glpA101::Mu d1* (9).

TABLE 2. Expression of *glpT* in $\Phi(glpA-lac)$ and $\Phi[glpA1(Oxr)-lac]$ strains

Strain	Genotype	G3P uptake (nmol/min per mg of protein) under the following growth conditions ^a :				β -Galactosidase activity (U) under the following growth conditions ^a :			
		+O ₂		-O ₂		+O ₂		-O ₂	
		-GF	+GF	-GF	+GF	-GF	+GF	-GF	+GF
ECL389	$\Phi(glpA-lac)$	0	22	0	31	16	54	14	420
ECL503	$\Phi[glpA1(Oxr)-lac]$	0	18	0	33	44	590	68	2,200

^a Cells were grown aerobically (+O₂) or anaerobically (-O₂) on oxylose medium. -GF, glycerol and fumarate omitted; +GF, glycerol and fumarate added.

inheritance of the desired mutation was confirmed by genetic linkages, cell growth, or enzyme assay.

Growth conditions. For routine cultures, LB medium (1.0% tryptone, 0.5% yeast extract, 0.5% NaCl) was used. For enzyme and transport assays, cells were grown at 37°C in a mineral medium (pH 7.0) buffered by 0.1 M phosphate (29) and supplemented with 0.03% casein acid hydrolysate (to prime growth). As the principal carbon and energy source, additional appropriate compounds were added. Aerobic cultures were grown with vigorous rotatory agitation and harvested in mid-exponential phase (approximately 100 Klett units; no. 42 filter). A control experiment showed that the specific activity of β -galactosidase in a $\Phi(glpA-lac)$ *glpR* strain remained almost constant when sampled every half hour from inoculation to stationary phase. Thus, expression of the *glpA* promoter appeared not to be growth phase dependent. Consequently, anaerobic cultures were usually grown to stationary phase in screw-cap test tubes filled to the top with medium and left undisturbed overnight. Anaerobic incubation of agar plates was carried out in sealed jars containing an atmosphere of H₂ and CO₂ (GasPak anaerobic system; BBL Microbiology Systems, Cockeysville, Md.). When used, fumarate was added to 20 mM; glucose, xylose, and potassium nitrate were added to 10 mM; glycerol was added to 0.2 mM (unless otherwise specified); cyclic AMP was added to 3 mM; KCN was added to 150 μ M; and tetracycline was added to 10 μ g/ml.

Enzyme assay. Cell extracts, prepared as described previously (8), were assayed for aerobic G3P dehydrogenase activity at 30°C by monitoring the reduction of MTT at 570 nm (16), and the specific activity was expressed in nanomoles per minute per milligram of protein. Protein concentrations were estimated with bovine serum albumin as the standard. β -Galactosidase activity was assayed in whole cells (rendered permeable by the addition of 1 drop of 0.1% sodium dodecyl sulfate and 2 drops of chloroform to the cell suspension) at 30°C by measuring the hydrolysis of *o*-nitrophenyl- β -D-galactoside at 420 nm, and the specific activity was expressed in units by the method of Miller (21).

Transport assay. Cells were suspended in mineral medium containing chloramphenicol (40 μ g/ml) for the assay of G3P

transport at 30°C as described previously (5), except that 5 ml of buffer was used to wash the cells at the end of the assay. Specific activity was expressed in nanomoles per minute per milligram of protein.

RESULTS

Mutant with a $\Phi(glpA-lac)$ operon resistant to aerobic repression. Strain ECL503 was selected from strain ECL389 [$\Phi(glpA-lac)$] as a spontaneous mutant which grew aerobically as a large colony on agar containing 5 mM lactose as the principal carbon and energy source and 0.2 mM glycerol as the inducer. The mutant showed increased aerobic expression of $\Phi(glpA-lac)$ when grown in minimal xylose medium (low catabolite repression) with glycerol as the inducer (8). When the mutant was used as a transduction donor to the lactose-negative strain ECL514 [$\Phi(glpA-lacZ^+Y)$] and selected for aerobic growth on lactose, all of the 48 transductants analyzed showed elevated aerobic activity levels of β -galactosidase, similar to that observed in the donor, when induced by glycerol. Thus, the regulatory mutation was tightly linked to the *glpA* locus. The aerobically induced level of β -galactosidase in the mutant ECL503 increased more than 10-fold over that of the parental strain. The anaerobically induced level also increased, albeit only about sixfold (8; this study). To learn whether the increased enzyme activity in the mutant was the result of gene amplification, we tested the activity of G3P permease encoded by the adjacent *glpT* operon. Whereas the aerobic and anaerobic induction of β -galactosidase in strain ECL503 increased in the expected manner, the induction pattern of G3P permease remained similar to that of the wild-type parent (Table 2). Thus, the mutation in question appeared to be a *cis*-regulatory change specifically affecting the expression of $\Phi(glpA-lacZ)$. The mutant fusion will be referred to as $\Phi(glpA1(Oxr)-lacZ)$ in analogy to the *frd(Oxr)* mutation we have previously reported (9). We next examined the effect of the mutation on the response of the fusion operon to several factors previously known to influence *glpA* expression.

Catabolite repression of $\Phi(glpA-lac)$ and $\Phi(glpA1(Oxr)-lac)$ expression in *glpR* background. Expression of the *glpA*

TABLE 3. Effects of glucose and cAMP on the constitutive expression of $\Phi(glpA^+-lac)$ and $\Phi[glpA1(Oxr)-lac]$

Strain	Genotype	β -Galactosidase activity (U) under the following growth conditions ^a :					
		+O ₂			-O ₂		
		NA	Glc	Glc + cAMP	NA	Glc	Glc + cAMP
ECL526	$\Phi(glpA-lac)$ <i>glpR</i>	83 (1.0)	34 (0.41)	73 (0.88)	380 (1.0)	230 (0.61)	270 (0.71)
ECL527	$\Phi[glpA1(Oxr)-lac]$ <i>glpR</i>	790 (1.0)	200 (0.25)	770 (0.97)	1,900 (1.0)	630 (0.33)	1,700 (0.89)

^a Cells were grown aerobically (+O₂) or anaerobically (-O₂) on xylose medium. NA, no addition; Glc, glucose added; Glc + cAMP, glucose and cAMP added. For each strain, data in parentheses give its relative enzyme activities normalized with respect to those found in cells grown anaerobically in the absence of glucose or cAMP.

TABLE 4. Effect of *fnr* mutation on the constitutive expression of $\Phi(\text{glpA-lac})$ and $\Phi[\text{glpA1(Oxr-lac)}]$

Strain	Genotype	β -Galactosidase activity (U) under the following growth conditions ^a :		Ratio of activities ($-\text{O}_2/+\text{O}_2$)
		$+\text{O}_2$	$-\text{O}_2$	
ECL526	$\Phi(\text{glpA-lac}) \text{ glpR}$	71 $\pm$ 5 (1.0)	400 $\pm$ 10 (1.0)	5.6
ECL538	$\Phi(\text{glpA-lac}) \text{ glpR fnr}$	69 $\pm$ 5 (0.97)	160 $\pm$ 10 (0.4)	2.3
ECL527	$\Phi[\text{glpA1(Oxr-lac)}] \text{ glpR}$	600 $\pm$ 40 (1.0)	2,100 $\pm$ 120 (1.0)	3.5
ECL539	$\Phi[\text{glpA1(Oxr-lac)}] \text{ glpR fnr}$	610 $\pm$ 50 (1.0)	1,600 $\pm$ 130 (0.76)	2.6

^a Cells were grown aerobically ($+\text{O}_2$) or anaerobically ($-\text{O}_2$) on xylose medium. Enzyme activities are averages of three experiments plus or minus the standard error. For each strain, numbers in parentheses are normalized with the isogenic *fnr*⁺ strain as 1.0.

operon is known to be significantly restrained by catabolite repression (19). This repression was compared in two isogenic *glpR* strains, ECL526 [$\Phi(\text{glpA-lac})$] and ECL527 [$\Phi[\text{glpA1(Oxr-lac)}]$], grown aerobically or anaerobically in xylose, xylose-glucose, or xylose-glucose-cAMP medium. The $\Phi(\text{glpA-lac})$ operon was subject to stronger catabolite repression aerobically than anaerobically (Table 3). Repression was partially relieved by the presence of cAMP. Catabolite repression of the $\Phi[\text{glpA1(Oxr-lac)}]$ operon was enhanced both aerobically and anaerobically. Interestingly, despite the enhanced repression, its reversal by cAMP was almost complete. This would suggest that the cAMP-catabolite gene activator protein (CAP) complex activated $\Phi[\text{glpA1(Oxr-lac)}]$ more effectively than it activated the parental $\Phi(\text{glpA-lac})$.

Effect of *fnr* mutation on expression of $\Phi(\text{glpA-lac})$ and $\Phi[\text{glpA1(Oxr-lac)}]$ in *glpR* background. Since the increased anaerobic expression of the *glpA* operon was previously shown to be partially dependent on activation by the Fnr protein, we also compared the effect of an *fnr* mutation on the expression of $\Phi(\text{glpA-lac})$ and $\Phi[\text{glpA1(Oxr-lac)}]$. In a *glpR* background, the *fnr* mutation lowered the expression of $\Phi(\text{glpA-lac})$ 2.5-fold (Table 4). In contrast, the expression of $\Phi[\text{glpA1(Oxr-lac)}]$ was lowered only 1.3-fold. As expected, under aerobic conditions the *fnr* mutation affected neither the expression of the parent fusion nor that of the mutant fusion. It should be noted, however, that even in the *fnr* background expression of both $\Phi(\text{glpA-lac})$ and $\Phi[\text{glpA1(Oxr-lac)}]$ was more than twofold higher anaerobically than aerobically. A separate redox control thus appeared to be functioning. The possibility that *fnr-1* is a leaky allele seems unlikely because this same allele did not allow appreciable anaerobic induction of the *nar* operon (11, 17).

KCN effect on $\Phi(\text{glpA-lac})$ and $\Phi[\text{glpA1(Oxr-lac)}]$ expression. The respiratory inhibitor KCN, known to inhibit cytochrome *o* activity (15), was observed to increase the

expression of $\Phi(\text{glpA-lac})$ in a *glpR*⁺ and *fnr*⁺ background (16). When we compared the effect of KCN (at a concentration still permitting growth) on isogenic *glpR* strains, the aerobic expression of both $\Phi(\text{glpA-lac})$ and $\Phi[\text{glpA1(Oxr-lac)}]$ was increased 1.5- to 1.7-fold in an *fnr*⁺ or *fnr* background (Table 5). Again, it seems that in addition to the FNR control, there exists a separate redox control.

Repression of $\Phi(\text{glpA-lac})$ and $\Phi[\text{glpA1(Oxr-lac)}]$ by nitrate respiration. Further evidence for an *fnr*-independent redox control for the expression of *glpA* came from the observation that under anaerobic conditions, nitrate respiration lowered the expression of $\Phi(\text{glpA-lac})$ about twofold (Table 6). This nitrate effect was diminished by a *narH* mutation, preventing the synthesis of a functional nitrate reductase complex, or a *narL* mutation, preventing nitrate reductase induction. The failure of the *narH* and *narL* mutations to block the nitrate effect completely was probably attributable to the activity of a second but minor nitrate reductase (2). Indeed, a *chlE* mutation, which prevented the synthesis of the molybdenum cofactor necessary for the catalytic activity of both the major and the minor nitrate reductases, completely abolished the nitrate effect. The nitrate effect therefore depended on its chemical reduction. A *narK* mutation almost abolished the nitrate effect (data not shown). No explanation can be offered at present, since the function of *narK* is still obscure. Although the expression of $\Phi[\text{glpA1(Oxr-lac)}]$ was no longer strongly repressed under aerobic growth conditions, its nitrate repression remained intact.

Independence of $\Phi(\text{glpA-lac})$ expression and dependence of $\Phi[\text{glpA1(Oxr-lac)}]$ expression on the *arc* regulatory system. It was recently found that numerous genes of aerobic function are under pleiotropic negative control by the products of *arcA* and *arcB* (7, 12). As expected, aerobic and anaerobic expression of $\Phi(\text{glpA-lac})$ was not affected by mutations in either of these regulatory genes (Table 7). The twofold anaerobic repression of the *glpD* operon encoding aerobic G3P dehy-

TABLE 5. Effect of KCN on aerobic expression of $\Phi(\text{glpA-lac})$ and $\Phi[\text{glpA1(Oxr-lac)}]$ in different *fnr* backgrounds

Strain	Genotype	β -Galactosidase activity (U) under the following growth conditions ^a :		Ratio of activities (+KCN/ -KCN)
		-KCN	+KCN	
ECL526	$\Phi(\text{glpA-lac}) \text{ glpR fnr}^+$	71 $\pm$ 5	120 $\pm$ 20	1.7
ECL538	$\Phi(\text{glpA-lac}) \text{ glpR fnr}$	69 $\pm$ 5	110 $\pm$ 30	1.6
ECL527	$\Phi[\text{glpA1(Oxr-lac)}] \text{ glpR fnr}^+$	600 $\pm$ 40	880 $\pm$ 40	1.5
ECL539	$\Phi[\text{glpA1(Oxr-lac)}] \text{ glpR fnr}$	610 $\pm$ 50	960 $\pm$ 70	1.6

^a Cells were grown aerobically on xylose medium. Activities are averages of three experiments plus or minus the standard error.

TABLE 6. Nitrate repression of $\Phi(\text{glpA-lac})$ and $\Phi[\text{glpA1(Oxr-lac)}]$

Strain	Genotype	β -Galactosidase activity (U) under the following growth conditions ^a :		Ratio of activities ($+\text{NO}_3^-$ / $-\text{NO}_3^-$)
		$-\text{NO}_3^-$	$+\text{NO}_3^-$	
ECL392	$\Phi(\text{glpA-lac})$	470 $\pm$ 10	260 $\pm$ 10	0.55
ECL560	$\Phi(\text{glpA-lac}) \text{ narH}$	460 $\pm$ 20	370 $\pm$ 10	0.80
ECL565	$\Phi(\text{glpA-lac}) \text{ narL}$	460 $\pm$ 20	320 $\pm$ 10	0.70
ECL566	$\Phi(\text{glpA-lac}) \text{ chlE}$	510 $\pm$ 10	570 $\pm$ 20	1.1
ECL519	$\Phi[\text{glpA1(Oxr-lac)}]$	2,500 $\pm$ 100	1,100 $\pm$ 50	0.44

^a Cells were grown anaerobically on xylose-fumarate-glycerol medium. Enzyme activities are averages of three experiments plus or minus the standard error.

TABLE 7. Differential effects of *arc* mutations on the expression of $\Phi(\text{glpA-lac})$ and *glpD*

Strain	Genotype	β -Galactosidase activity (U) ^a			G3P dehydrogenase activity (U) ^a		
		+O ₂	-O ₂	Ratio (-O ₂ /+O ₂)	+O ₂	-O ₂	Ratio (-O ₂ /+O ₂)
ECL526	$\Phi(\text{glpA-lac})$ <i>glpR</i>	61	360	5.9	47	17	0.4
ECL597	$\Phi(\text{glpA-lac})$ <i>glpR arcA</i>	60	320	5.3	48	49	1.0
ECL598	$\Phi(\text{glpA-lac})$ <i>glpR arcB</i>	56	280	5.0	48	53	1.1

^a Cells were aerobically (+O₂) or anaerobically (-O₂) grown on xylose medium.

drogenase, however, was completely lifted by either an *arcA* or an *arcB* mutation.

DISCUSSION

Results from this study showed that the opposite changes of *glpD* and *glpA* expression were caused by different mechanisms. The decreased anaerobic expression of *glpD* resulted from repression by the pleiotropic *arcA* gene product, which appeared to receive the respiratory signal from the *arcB* product (7, 12). The increased anaerobic expression of *glpA*, on the other hand, appeared to be the sum result of two controls, an FNR-dependent mechanism and a second mechanism that was sensitive to the redox state. The existence of such a redox mechanism was also indicated by previous observations that in the presence of an *fnr* mutation, including a deletion, the anaerobic expression of the $\Phi(\text{frdA-lacZ})$ was still repressed two- to threefold by aerobic growth (13, 25). The nature of the second redox control mechanism remains to be discovered. It can be imagined that the [ubiquinone]/[ubiquinol], for example, serves as a signal for the control, since this electron adaptor serves in both aerobic respiration and anaerobic nitrate respiration (6).

The second redox control mechanism might also be involved in the regulation of the formate hydrogen lyase operon (*fdh*). This operon is repressed by either oxygen or nitrate. Nitrate repression is relieved to various extents by insertion mutations in the *narK*, *narGHJI*, or *narL* operon (27). Expression of the *fdh* operon, however, is dependent on *ntrA* (*glnF*) and not on *fnr* (1). The *glpA* operon does not have such a consensus sequence (3) for the NtrA protein, and an *ntrA::Tn10* mutation did not have a significant effect on $\Phi(\text{glpA-lac})$ expression (data not shown).

The FNR protein is highly homologous to CAP (23, 24). It is of interest that whereas the dependence of $\Phi[\text{glpA1}(\text{Oxr-lac})]$ expression on FNR was decreased, the dependence on CAP appears to be increased. It is tempting to speculate that in the wild-type cell, low aerobic expression of *glpA* largely reflects activation by CAP, whereas the high anaerobic expression of the operon largely reflects activation by the FNR protein.

As a set of genes specifying a catabolic network, the *glp* regulon is unusually complex in that its structural genes are controlled in *trans* by multiple regulatory elements. In addition to the specific control by the *glpR* product, expression of all the structural genes is influenced to various degrees by the *crp* product (CAP). Expression of the *glpD* operon can be further adjusted by the *arcA* product, and expression of the *glpA* operon can be further adjusted by the *fnr* product. Thus, according to a term we have proposed (12), among genes of the *glp* regulon there are members of at least three higher systems of regulation, the *crp*, *fnr*, and *arc* modulons. To account completely for the respiratory control of the *glpA* operon, it seems that yet another pleiotropic control system must be identified.

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